REMARKS

The claims have not been amended but are included for convenience.

The only remaining issues are whether claims 1, 2, and 12 - 22 meet the non-obviousness requirement of 35 USC 103 over the new combination of Richardson, Amoss US 4072668 and DavisUS 6150354. It is submitted that they do. The applicant appreciates the examiner's agreement with her arguments that the present invention is not obvious in view of the previously cited combinations of Saiko and Davis or of Walles, Yorke, Trubinkova and Davis .

As noted previously, the present invention involves a central activity involving stimulation of the hypothalmic-pituitary-gonaodal axis by administering a centrally-acting acetylcholinesterase inhibitor at the beginning of the follicular phase. In humans this commences about fourteen days prior to the release of an egg. After this administration, the claim requires determining whether a normal follicular response has been obtained and deciding on further administration of said compound based on the results of said determination

The Examiner's references are noted. The Richardson reference is cited in our original disclosure, in the fourth paragraph.

> "As cholinergic agents have no direct effect on anterior pituitaries to stimulate LH or FSH release, they were presumed to release gonadotropin-releasing hormone (GnRH, formerly thought to be two hormones, luteinizing hormone releasing hormone, LHRH, and follicle stimulating hormone releasing hormone, FSHRH) from the hypothalamus. Since the advent of assays for GnRH, this fact has been directly demonstrated. (Richardson SB, Prasad JA, Hollander CS, Acetylcholine, melatonin, and potassium depolarization stimulate release of luteinizing hormone-releasing hormone from rat hypothalamus in vitro. Proc Natl Acad Aci U S A 79(8):2686-9, 1982)."

The ability of LHRH or LRF to stimulate ovulation - even in humans, not necessarily lower "mammalians" is described in the second paragraph of the "Detailed Description of the Invention" section

> "GnRH and various longer acting agonists are used in a wide variety of clinical situations. They may be used to stimulate or suppress the HPG axis. They may be used alone, or in combination with gonadotropic preparations, hormones, or hormone antagonists. Short-term (or pulsatile) administration of GnRH stimulates gonadotropin secretion, while continuous administration or the use of long acting analogs for more than approximately two weeks desensitizes the gonadotropes and decreases gonadotropin secretion. This "medical castration" has numerous applications. (Ascoli M and Segaloff DL, Adenohypophyseal hormones and their hypothalamic releasing factors, in Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th edition, McGraw-Hill, New York, 1996, pp 1363-1382)"

Thus, the facts that the Examiner cites could have been combined to suggest that anticholinesterases be used to stimulate the hypothalamic-pituitary-gonadal axis in humans, but, to the best of our knowledge, no one did so. The present inventor's insight in realizing that the combination of these facts provided a means for treatment of failure of ovulation is inventive and not obvious. Most inventions involve combinations of what is old. Invention lies in putting them together in a new and useful way. This is what has been done here.

In fact been demonstrated in humans that infusion of the cholinesterase inhibitor, physostigmine, even in amounts sufficient to cause nausea and vomiting, failed to change LH levels. (Davis B, et al, Psychoneuroendocrinology 1982; 7(4):347-354) copy enclosed.

It was not clear to Richardson et al that their *in vitro* findings in male tissue could be applied even to intact female animals, not to mention humans. Their demonstration that atropine could not

block stimulation of LHRH in vitro was inconsistent with other published data that atropine could block ovulation in intact animals. In their explanation for this inconsistency, the authors explain that

> "The failure of atropine to block LH-RH stimulation does not provide an explanation for the ability of atropine to block ovulation. However, our findings with atropine *in vitro* in male rats would not be expected to be predictive of *in vivo* findings in females, in whom regulation of LH-RH release is obviously different and dependent upon fluctuant levels of estrogen, progesterone, and other hormones during the menstrual cycle.

Adding to the uncertainty of translating an effect from a petri dish to an intact animal was the concentration of neostigmine used. The LRF effect was shown at 1 μM neostigmine. The IC50 of neostigmine for acetylcholinesterase inhibition in the rat is 0.11 μM. (Ueki et al, JPET 1993; 264(1):152-157) Therefore, its acetylcholinesterase would have been overwhelmingly inhibited by 1 μM neostigmine. It would have been doubtful that such a dose could be administered to living animals.

The present application as filed provides information, albeit unreferenced, that cholinergic agents could be given to living animals in doses capable of releasing LH. The second and third to the last sentences in the third paragraph in the Background of the Invention section, read "In intact rats, injection of acetylcholine into the lateral ventricle stimulates LH release. This can be blocked with atropine or enhanced with prostigmine." Thus the disclosure teaches that cholinergic stimulation of LH release can be done in a living animal, and can be done with the participation of the cholinesterase inhibitor, prostigmine (also called neostigmine).

However, the data most directly indicating cholinergic stimulation of the hypothalamic-pituitary-reproductive axis were obtained in beagle dogs and submitted to the Patent Office in our response of December 1, 2008. Thirty-nine dogs who received 2 to 10 mg/kg of galantamine for 6 months to a year demonstrated pronounced dose-related increases in ovarian weight, and developed ovarian cysts

and endometrial hyperplasia. The total daily exposure to galantamine in the dogs was comparable to that achievable in human use.

It is therefore submitted that the present claims meet the requirements of 35 USC 103 and should be allowed. An early action to this effect is respectfully solicited.

Respectfully submitted,

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